Fig. 2.—Man (adult). Photomicrograph of part of a Transverse Section through the Posterior End of the Posterior Commissure, showing the Irregular Proximal (posterior) part of the Mesocœlic Recess. × 58.

Fig. 3.—Man (adult). Photomicrograph of part of a Transverse Section through the Posterior Part of the Posterior Commissure at a level slightly anterior to that of the section represented in fig. 2, to show the terminal (anterior) portion of the mesocœlic recess, with its characteristic columnar epithelium. × 300.

Explanation of Lettering.

b. v., blood vessels ; gl., globular bodies ; m. r., mesocœlic recess ; p. c., posterior commissure ; s.c. o., sub-commissural organ.

Autotoxæmia and Infection.

By E. C. Hort, F.R.C.P. Ed., Assistant Physician to the Italian Hospital.

(Communicated by William Osler, F.R.S. Received June 2,—Read June 23, 1910.)

The object of this communication is to show :—

That fever,* loss of weight, and a rise in the antitryptic values of the blood serum, three results common to infection in man, can be reproduced in animals by the subcutaneous injection of small quantities of distilled water.

Control Observations.

The following precautions were found to be necessary in order to demonstrate these effects, apart from selection of healthy animals for injection, and observance of strict aseptic conditions as to site of injection, instruments used, and water injected :—

Fever.

1. Selection and repeated testing of thermometers of guaranteed accuracy.
2. Accurate approximation of the temperature of the water injected to the temperature of the animal receiving the injection.
3. Determination of the mean average, and of the upper normal limit of, temperature of the species of animal selected for injection.
4. Demonstration that repeated handling and the taking of repeated thermometric observations do not cause a rise of temperature in the control animals.
5. Demonstration that mere subcutaneous puncture, or injection of air equivalent in bulk to the volumes of water injected, does not cause fever in the control animals.

* By fever is here meant a rise of temperature, and nothing more.

Loss of Weight.

6. Selection of a species of animal, such as the guinea-pig, on which reliable observations as to weight can be made for several days previous and subsequent to injection in order to establish necessary control.

7. Exclusion of error owing to any loss of weight being due to refusal of the animals to take food after injection.

Rise in Antitryptic Values of Serum.

8. Demonstration of the accuracy of the method used to measure antitryptic values.

9. Demonstration of strict parallelism between the antitryptic values of the serums of normal individuals of the species of animal injected.

The precautions requiring further detail are those numbered 3, 4, 5, 7, 8, 9.

3. The mean average temperature of 135 guinea-pigs selected for experiment was 101·4° F., or 38·6° C., the upper normal limit of temperature being 102·2° F., or 39° C. The mean average temperature of 50 apparently healthy rabbits was 102·4° F., or 39·1° C., the upper normal limit of 103° F., or 38·6° C., usually assigned, being accepted.

4. Repeated handling *per se* was invariably found to produce no rise of temperature in healthy guinea-pigs or rabbits. Unless, however, great gentleness be employed in taking repeated thermometric observations on the guinea-pig, fever is easily induced, owing to slight abrasion of rectal mucous membrane caused by insertion of the thermometer and consequent septic absorption. This does not occur in the rabbit with ordinary care. Hence the effect of multiple injections involving a series of thermometric observations extending over several days can only be satisfactorily studied in the rabbit. This does not apply to the study of the effects of single injections in the guinea-pig, as all observations can be completed in one day. After injury to rectal mucous membrane there is always a latent period of not less than 12 hours before fever caused by introduction of the thermometer appears, whereas the fever caused by injection of water is never more than five hours in reaching its maximum after injection. Hence in animals controlling injections on the first day a rise of temperature is always absent, provided that the animal is healthy.

5. Neither subcutaneous puncture nor injections of air up to 10 c.c., the maximum quantity of water injected into guinea-pigs in these experiments for the demonstration of fever, cause any rise of temperature in guinea-pigs.

7. The loss of weight observed in guinea-pigs after multiple injections of water can be shown not to be due to refusal of the animals to take food,

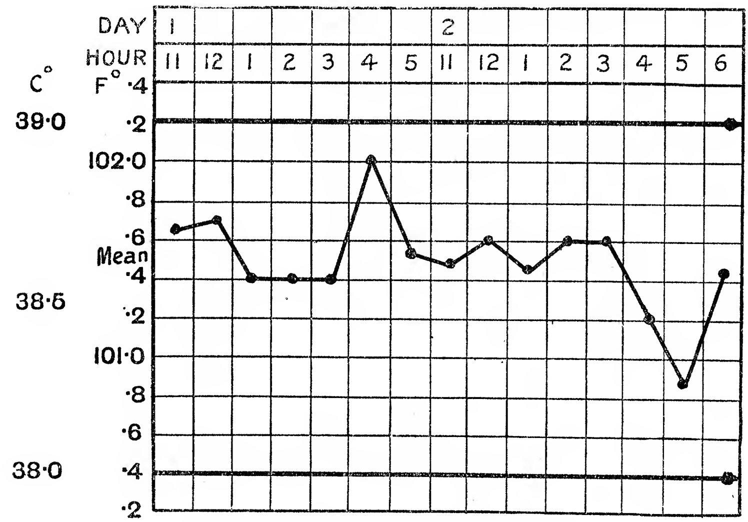
if they are kept in separate cages, and if the amount of food supplied and devoured be carefully noted.

8. *Vide* heading below, " Antitryptic values."

9. *Vide* heading below, " Antitryptic values."

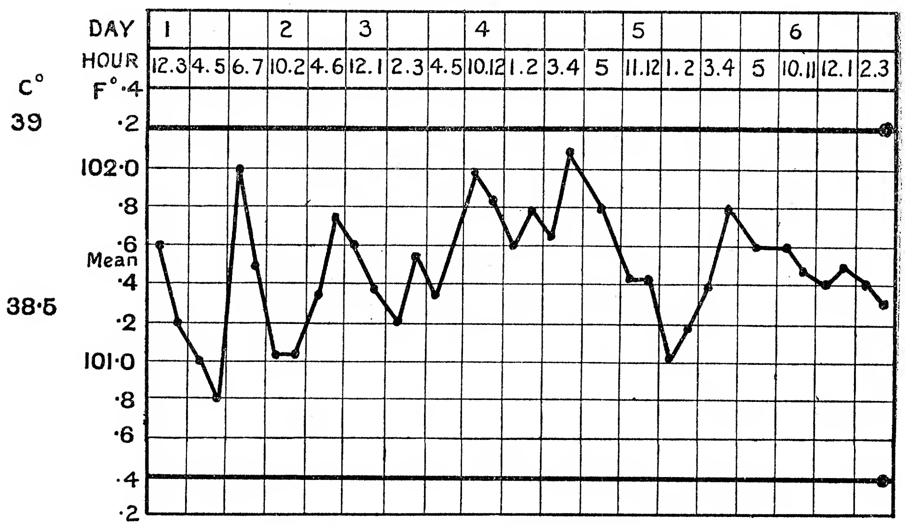
In order to represent in a graphic form the necessary control observations, the actual charts are subjoined showing absence of fever in uninjected animals.

CHART 1.—Guinea-pig. (Control.)



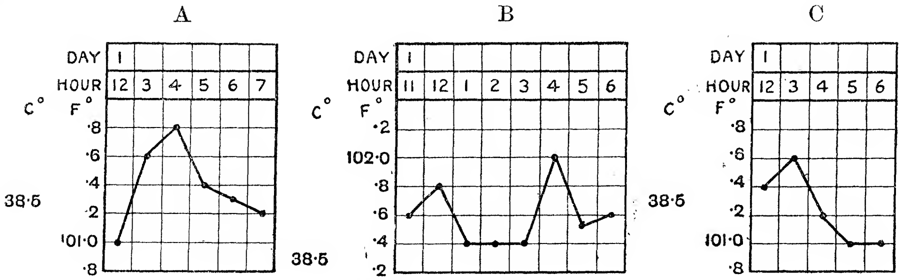
Upper normal limit = 102·2° F., 39° C.

CHART 2.—Guinea-pig. (Control.)

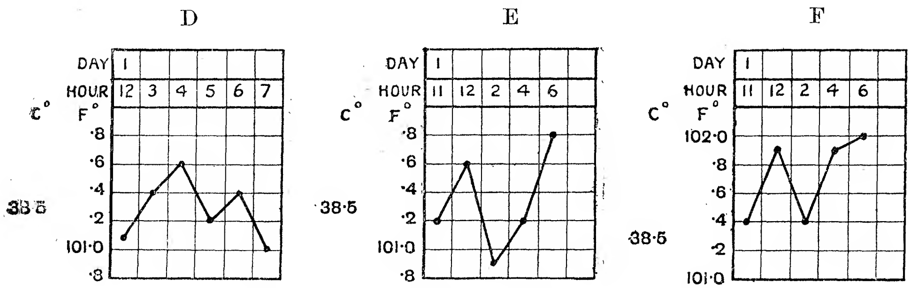


Upper normal limit = 102·2° F., 39° C.

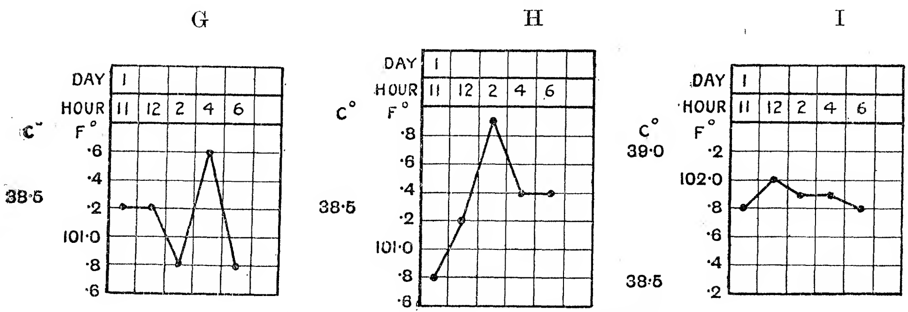
Chart 3.—Guinea-pigs. (Controls.)



Normal upper limit = 102·2° F., 39° C.



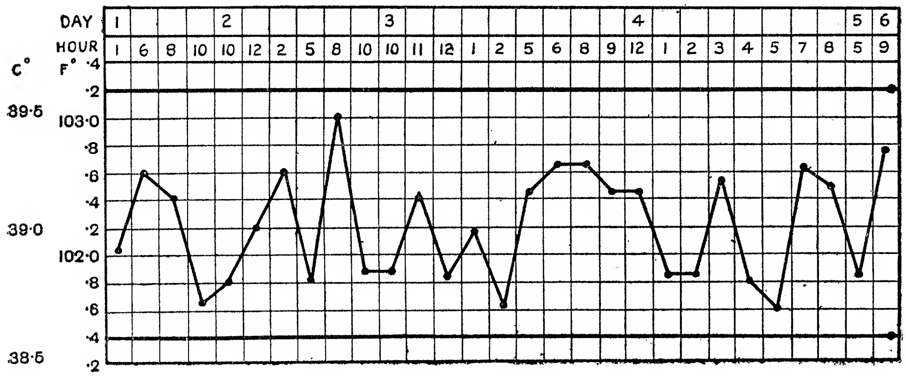
Normal upper limit = 102·2° F., 39° C.



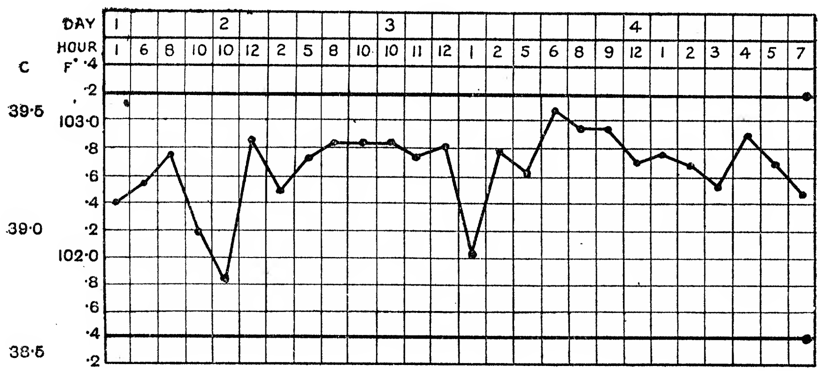
Normal upper limit = 102·2° F., 39° C.
Mean = 101·5° F., 38·6° C.

CHART 4.—Rabbit. (Control.)

A



B



EXPERIMENTS.

The results of injection of water were as follows :—

A. *On Temperature.*

1. *Single Injections.*—Sixty guinea-pigs, varying in weight from 100 to 600 grammes, were injected under the skin of the abdomen with single injections of boiled distilled water, in quantities varying from 1 c.c. to 10 c.c. A rise of temperature was observed in 50. It has so far not been possible to establish a constant between the bulk of injection, the weight of the animal injected, and the degree of fever produced. A marked rise has occurred after injection of a quantity of water equivalent to 1/304 of the body weight. The rise in temperature after single injections is rapid and fugitive. It attains its maximum in from 2 to 5 hours. A rise above 103° F. or 39·5° C. after first injections is exceptional, and is often less. The rise is easily missed unless repeated observations are taken, at least once an hour. Twelve rabbits

received subcutaneous injections of water in quantities varying from 10 to 60 c.c. With the former quantity a slight rise occurs, even in rabbits weighing $2\frac{1}{2}$ kilogrammes, with the latter a marked rise, sometimes to 104·5° F. or 40·3° C. Some elevation of temperature above the normal occurred in all 12 animals. An injection of 23 c.c. into a marginal vein of the ear of a rabbit weighing $1\frac{1}{2}$ kilogramme caused a rise to 106° F., or 41·1° C. The rise in rabbits shows roughly the same abruptness, the same length of time to reach the maximum, and the same fugitive nature as in guinea-pigs.

2. *Multiple Injections.*—The effect of multiple injections in both rabbit and guinea-pig is to cause a continuous fever lasting only so long as the injections are continued, abruptly ceasing as soon as the injections are stopped. Multiple injections were given to 30 guinea-pigs and 10 rabbits, and caused fever in every case. To several other guinea-pigs than these 30 injections larger than 10 c.c. were given, and instead of a rise of temperature a profound fall followed. The same effect can be produced by giving small injections, closely spaced. On correct spacing of two injections into the same animal hypersensitisation is frequently induced, within limits not yet determined, but clearly evidenced by the rise of temperature induced by the second injection being greater than that induced by the first. Owing to the difficulty already mentioned of repeated thermometric observations extending over several days in the guinea-pig, study of the effect of multiple injections of more than two in number is unsatisfactory, but the effects of hypersensitisation in this animal after two injections are convincing. For study of the effects of injections over a series of days the rabbit must be used.

It is impossible to reproduce all the very numerous charts illustrating the induction of fever after both single and multiple injection in the rabbit and guinea-pig, but a few typical charts are subjoined for comparison with the control charts given above.

CHART 5.—Guinea-pig.

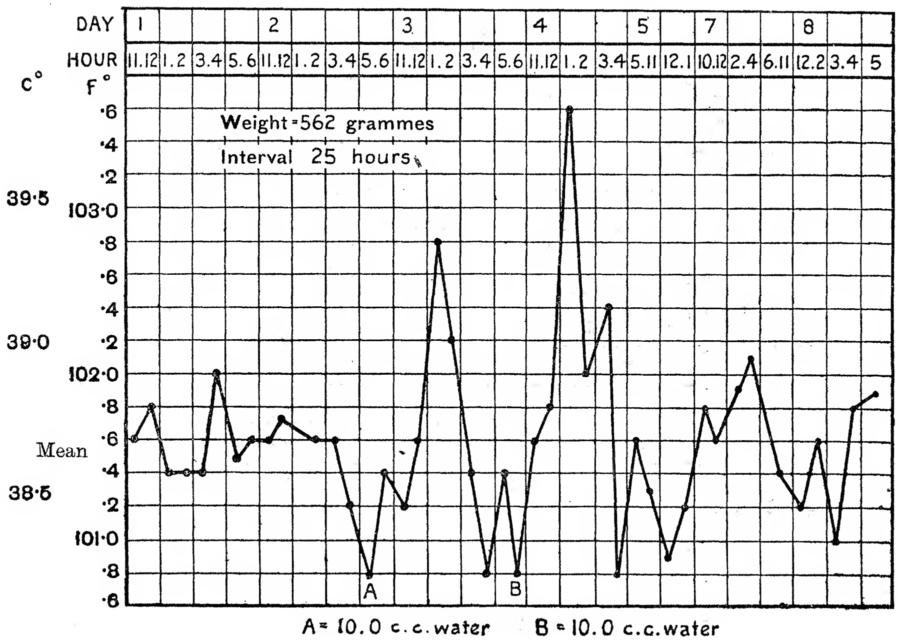


CHART 6.—Guinea-pig.

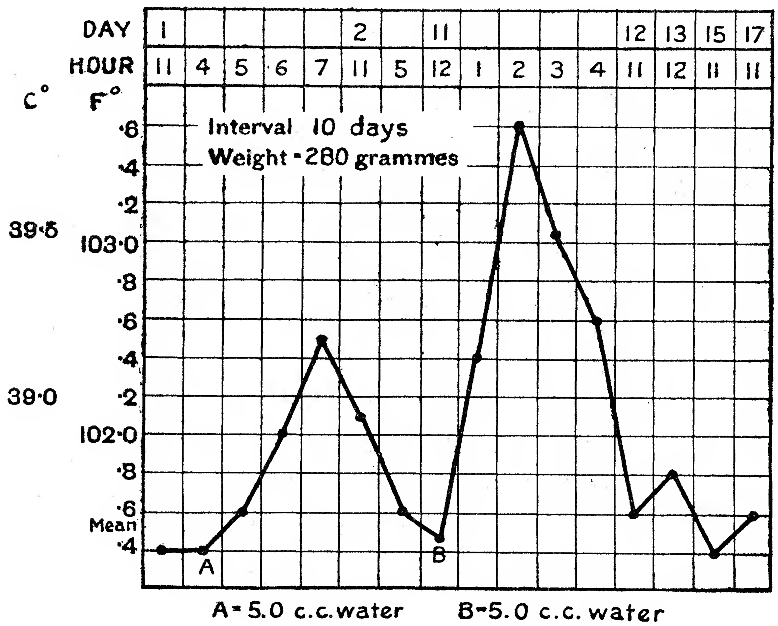


Chart 7.—Guinea-pig.

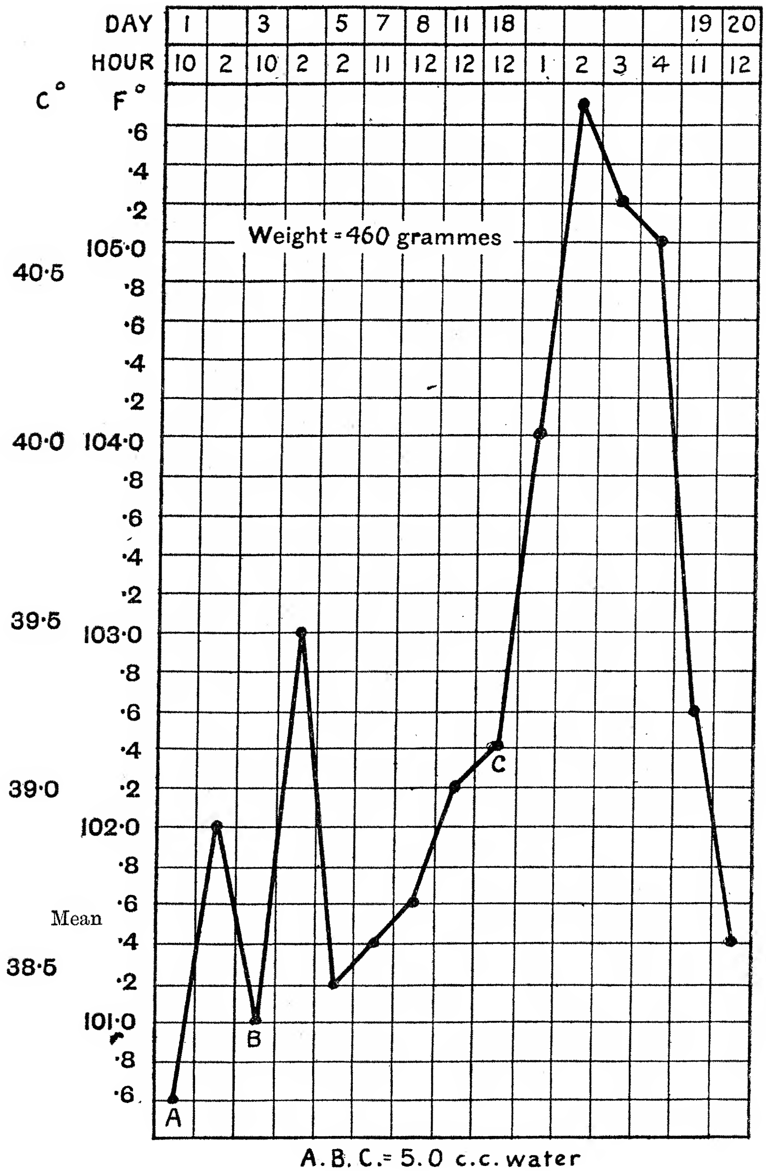


CHART 8.—Rabbit.

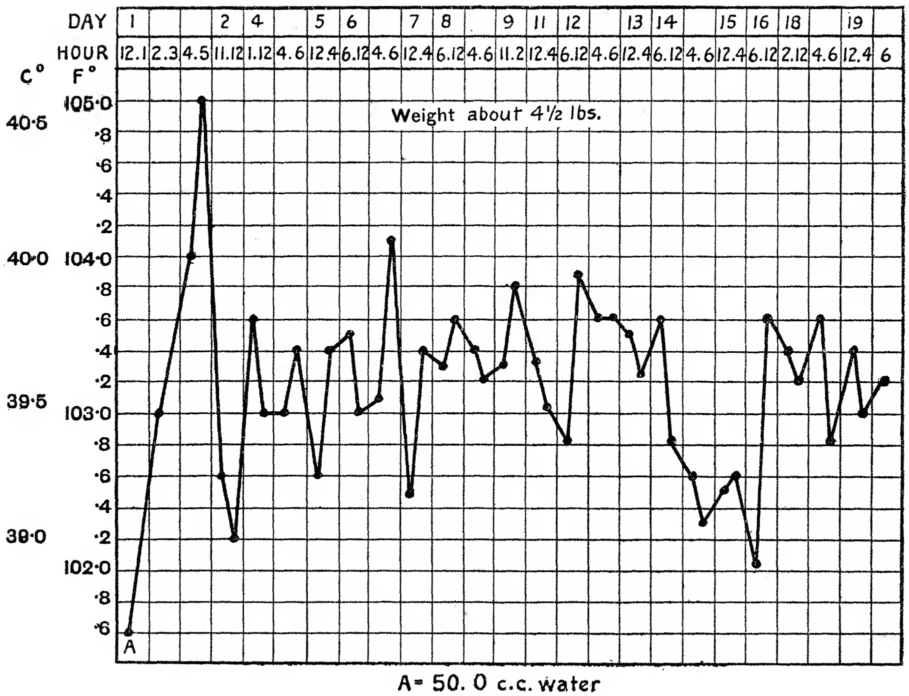
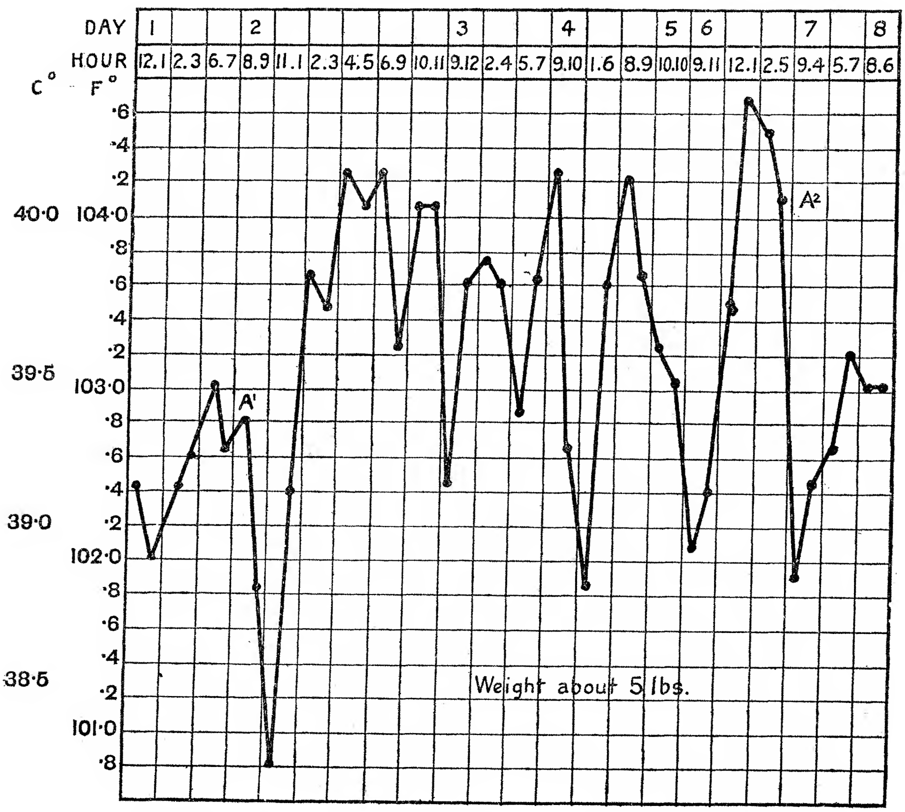


Chart 9.—Rabbit.



A¹ = first injection, 40 c.c. Twelve injections in all, with a total bulk of 246 c.c. from A¹ to A².

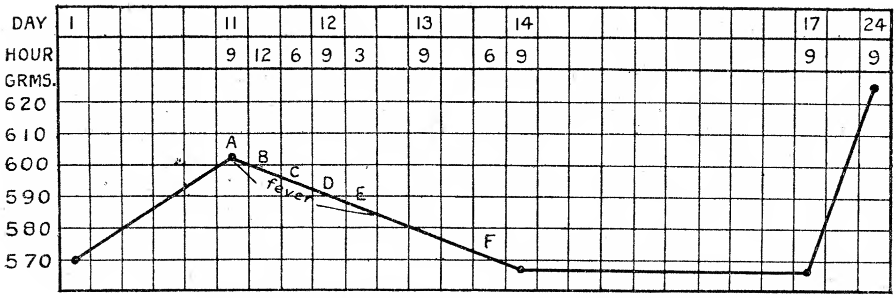
B. *On Body Weight.*

Observations on variations in the weight after single small injections of water into the guinea-pig were inconclusive.

The results on weight of multiple injection are illustrated by the subjoined charts, which are self-explanatory.

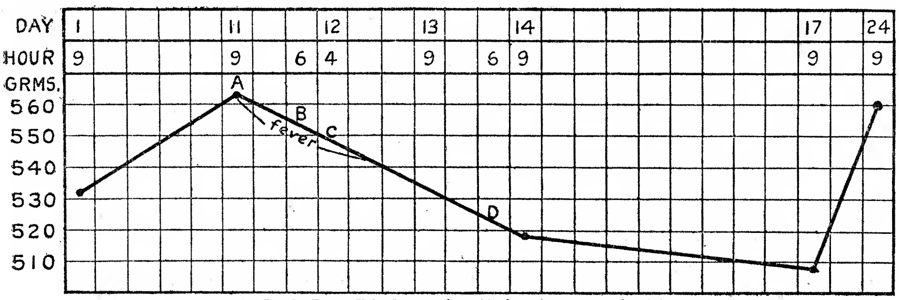
CHART 10.

A.



A.B.C.D.E.F = 100.0 c.c. in divided doses in 33 hours

B.



A.B.,C,D. = 70.0 c.c. in divided doses in 33 hours

C. *On the Antitryptic Values of the Serum.*

(a) *Rabbits.*

Twenty cubic centimetres of water were injected subcutaneously, in four divided doses, into a healthy rabbit. The animal was bled immediately before the first injection, and 60 minutes after the last injection, to the extent of 5 c.c. on both occasions. A control animal was bled at the same times, but was not injected. Estimation of the antitryptic values of all four serums was made the following morning. In order to ensure accuracy of observation in estimation of the antitryptic values of the serums two methods were employed: (1) The chemical method of Sörensen[1] for estimating the velocity of action of a trypsin-casein mixture (by an adaptation of which I had shown that it was possible[2] to determine variations in the antitryptic values of the serum of subjects of malignant disease with sufficient accuracy to aid materially in diagnosis). (2) The viscosity method introduced by Spriggs for estimating the velocity of a trypsin digestion adapted by Golla[3] for determining antitryptic values of serum in normal and pathological

conditions. The results given by both methods in determining the values of the serums after injection of water were parallel and mutually confirmatory. The results of the viscosity method, slightly modified by myself, being the simplest, are given below. To 10 c.c. of a 40-per-cent. mixture of casein and water is added 0·25 c.c. of the serum of the antitryptic value of which estimation is desired. At a fixed time 1 c.c. of a 1-per-cent. filtered fresh solution of trypsin in water is added to the casein mixture, and digestion allowed to proceed at a constant temperature, in this case 19° C. Before adding the trypsin solution, observations are taken with a stop-watch to determine the length of time occupied by the discharge from the viscosimeter of the contents of the bulb between two marked points. After addition of the trypsin solution, repeated observations are taken at intervals in exactly the same way. All the serum-casein-trypsin mixtures are then put through the viscosimeter at the same intervals of time, and in this way comparable results are obtained.

Experiment.

	Injected rabbit before first injection.	Injected rabbit after last injection.	Control rabbit before time of first injection.	Control rabbit after time of last injection.
Discharge time before addition of trypsin	2.49	2.49$\frac{1}{5}$	2.49$\frac{1}{5}$	2.49
First reading, 16 minutes after addition of trypsin	2.12	2.12$\frac{3}{5}$	2.12	2.11$\frac{3}{5}$
Second reading, 45 minutes after addition of trypsin	2.0	2.1$\frac{1}{5}$	2.0$\frac{1}{5}$	1.59$\frac{3}{5}$
Third reading, 110 minutes after addition of trypsin	1.50$\frac{1}{5}$	1.51$\frac{4}{5}$	1.50$\frac{1}{5}$	1.50

From these figures it is clear that, whereas, in the case of the control, the serum from the second bleeding showed, if anything, a somewhat lower antitryptic value than the serum from the first bleeding, the serum of the injected animal showed after injection a slight but unmistakable rise in antitryptic values compared with the reading before injection, in spite of the smallness of the injection. The weight of the animal was almost 5 lbs.

(b) *Guinea-pigs.*

A guinea-pig, weighing 442 grammes, received subcutaneously an injection of 5 c.c. of water. This was followed in three hours by a rise of temperature, the height of which was intensified by a previous injection of the same bulk 16 days previously, and by a third injection of the same bulk 18 days previously. The final injection was followed by a fall of weight from 442 grammes to

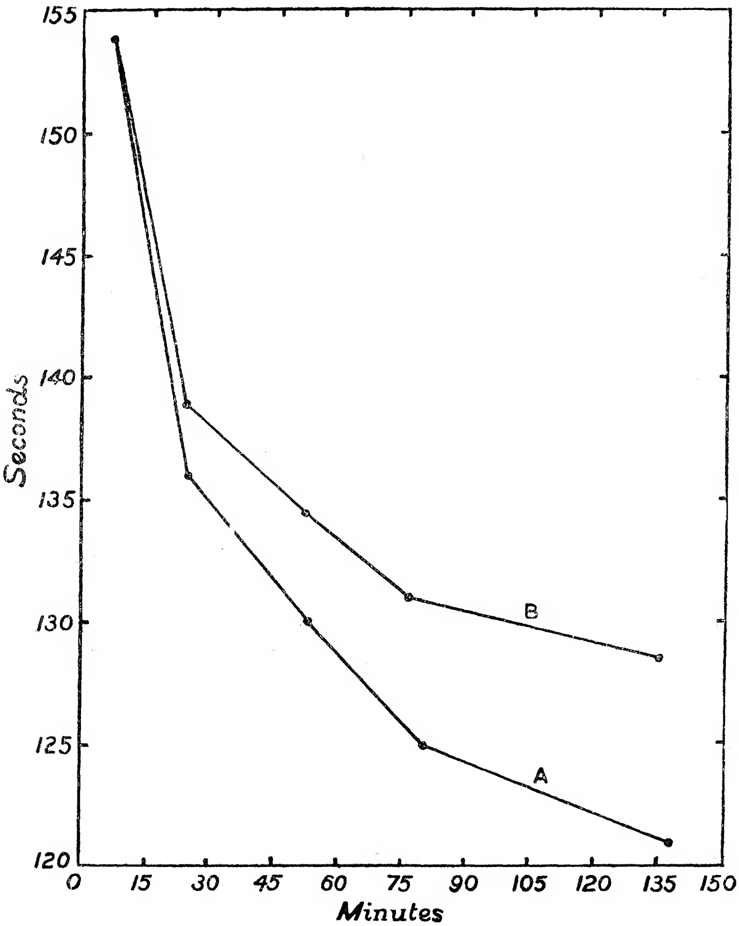
429 grammes. Four days after the injection the animal was killed and estimation made of the antitryptic values of the serum. The estimations were made in duplicate. Temperature, 18° C. throughout. Quantities as before.

Experiment.

	Control 1A.	Control 1B.	Injected animal 2A.	Injected animal 2B.
Discharge time before addition of trypsin	3. 19$\frac{3}{5}$	3. 19$\frac{2}{5}$	3. 20	3. 20
First reading, 6 minutes after addition of trypsin	2. 34$\frac{2}{5}$	2. 34$\frac{3}{5}$	2. 34$\frac{3}{5}$	2. 34$\frac{4}{5}$
Second reading, 35 minutes after addition of trypsin	2. 16	2. 16$\frac{2}{5}$	2. 19	2. 18$\frac{2}{5}$
Third reading, 65 minutes after addition of trypsin	2. 10	2. 10$\frac{2}{5}$	2. 15	2. 14$\frac{4}{5}$
Fourth reading, 95 minutes after addition of trypsin	2. 5$\frac{1}{5}$	2. 5$\frac{2}{5}$	2. 11$\frac{3}{5}$	2. 11$\frac{2}{5}$
Fifth reading, 155 minutes after addition of trypsin	2. 2	2. 2$\frac{1}{5}$	2. 8$\frac{2}{5}$	2. 8$\frac{1}{5}$

These results may be expressed in the form of a curve (p. 542), from which it will be seen that in this case the effect of multiple injections of water was to give rise to a high antitryptic value in the serum of the injected animal, represented at the maximum point of divergence by approximately six seconds.

Viscosity Curve showing Antitryptic Power of Serum after Injection of Water. All four serums at same point six minutes after commencement of digestion.



The validity of these last figures rests on demonstration that the serums of healthy animals of the same species give closely parallel antitryptic values. This I have been able to show in the case of the horse, rabbit, and guinea-pig, by numerous observations as yet unpublished. This has already been shown for man.[4] The serums of two healthy guinea-pigs, taken at random, gave the following readings. The estimations were done in duplicate. Temperature, 16° C. throughout. Quantities as before :—

	Animal 1A.	Animal 1B.	Animal 2A.	Animal 2B.
Discharge time before addition of trypsin	3. 15$\frac{3}{5}$	3·15	3. 16$\frac{2}{5}$	3. 15$\frac{4}{5}$
First reading, 6 minutes after addition of trypsin	2. 50$\frac{4}{5}$	2. 51$\frac{1}{5}$	2. 51$\frac{2}{5}$	2. 51$\frac{3}{5}$
Second reading, 35 minutes after addition of trypsin	2. 39	2. 39	2. 39$\frac{3}{5}$	2. 39
Third reading, 65 minutes after addition of trypsin	2. 30$\frac{2}{5}$	2. 30$\frac{2}{5}$	2. 30$\frac{4}{5}$	2. 30$\frac{4}{5}$
Fourth reading, 95 minutes after addition of trypsin	2. 27$\frac{1}{5}$	2. 27	2. 27$\frac{3}{5}$	2. 27$\frac{2}{5}$

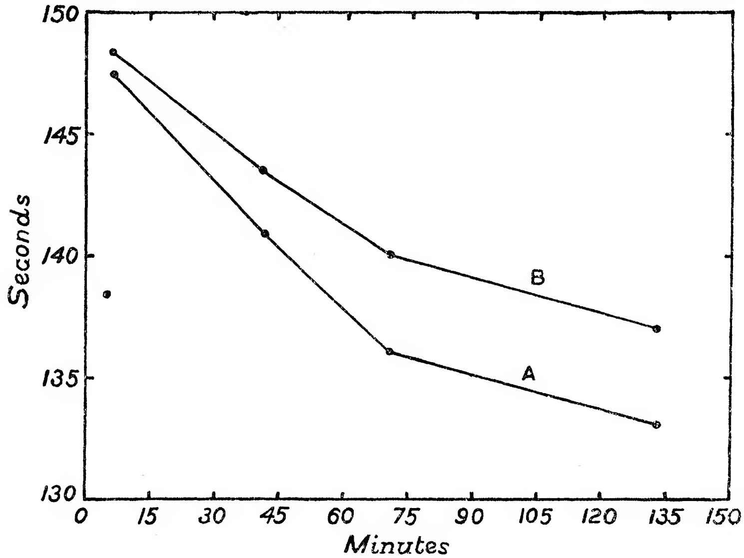
The difference between the means of the two duplicates of the two serums is not in excess of three-fifths of a second, once digestion has begun.

Comparison of the following curve (p. 544) with the preceding curve illustrates the gross resemblance that exists between the curves of animals that have received injections of water only, and of those that have been artificially infected with bacterial products.

A guinea-pig of standard weight was injected on Saturday, October 23, 1909, at 10 A.M., with 0·91 of a M.L.D. of diphtheria toxin. On the following Monday, at 10 A.M., he was killed, and the antitryptic value of the serum estimated the following day. Estimations in duplicate. Temperature, 12° C. throughout.

	Control 1A.	Control 1B.	Injected animal 2A.	Injected animal 2B.
First reading, 15 minutes after addition of trypsin	2. 28$\frac{3}{5}$	2. 28$\frac{2}{5}$	2. 29$\frac{4}{5}$	2. 29$\frac{1}{5}$
Second reading, 45 minutes after addition of trypsin	2. 21$\frac{1}{5}$	2. 21$\frac{2}{5}$	2. 24$\frac{3}{5}$	2. 24$\frac{1}{5}$
Third reading, 75 minutes after addition of trypsin	2. 16$\frac{3}{5}$	2. 16$\frac{4}{5}$	2. 20$\frac{2}{5}$	2. 20
Fourth reading, 135 minutes after addition of trypsin	2. 13	2. 13$\frac{1}{5}$	2. 17$\frac{2}{5}$	2. 17

Viscosity Curve showing Antitryptic Power of Serum after Injection of Diphtheria Toxin.



A, Serum of control animal, mean of duplicates.
B, Serum of injected animal, mean of duplicates.

Conclusions.

These experiments show that, as regards gross results, there is a close parallelism between :—

(1) The temperature charts of animals that have received injections of water and of those that have received injections of living bacteria, or of solutions of bacterial toxins.

(2) The weight curves of animals that have received multiple injections of water and of those that have received injections of living bacteria, or of solutions of bacterial toxins.

(3) The antitryptic values of the serums of animals that have received multiple injections of distilled water, or of solutions of bacterial toxins.

They lend strong support, in fact, to the view I have already advanced,[5] based on clinical observation, that however great the share taken by micro-organisms, including protozoa, and their products in initiating the disease complex of infection, the net result is, perhaps to a large extent, a state of true auto-intoxication.

Note.—The only observations on the effect of subcutaneous injection of small quantities of distilled water as regards the production of fever that I can find in the literature are those of Krehl in 1897, who specifically denies,

as a result of his observations, that the injection of distilled water by the subcutaneous route can cause fever.[6] In the paper referred to it is clear that neglect to make hourly observation after injection is responsible for his statement.

REFERENCES.

1. Sörensen, "Etudes enzymatiques Lab. de Carlsberg," 1907, vol. 7, p. 1.
2. Hort, "Diagnosis of Cancer by Examination of the Blood," 'Brit. Med. Assoc. Proc.,' Belfast, July, 1909.
3. Golla, "The Antitryptic Index," 'Brit. Med. Assoc. Proc.', Belfast, July, 1909.
4. Hort, *vide* 2.
5. Hort, 'Roy. Soc. Med. Proc.,' June, 1909.
6. Krehl, L., "Versuche über die Erzeugung von Fieber bei Thieren," 'Archiv f. exper. Path. u. Pharm.,' 1895, vol. 35, pp. 222—268.

The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-pigs, and Mice, and its Relationship to the Body Weight and to the Surface Area expressed in a Formula.

By Georges Dreyer, M.A., M.D., and William Ray, B.Sc., M.B.

(Communicated by Prof. F. Gotch, F.R.S. Received May 5,—Read June 23, 1910.)

(Abstract.)

The blood volume of animals has for many years been the subject of numerous investigations. This is but natural, considering its great importance for the study of disease. As, however, the results obtained are very discordant, we have determined the blood volumes of rabbits, guinea-pigs, and mice by Welcker's method, by washing out the circulatory system, and by following the percentage fall of hæmoglobin after bleeding.

Our experiments have given the following results:—

(1) The blood volume of living mammals can be determined very accurately by bleeding the animal (about 20 per cent. of its original blood volume) and determining the percentage fall of hæmoglobin at the moment when equilibrium is reached. This method gives results remarkably concordant with those obtained by washing out the circulatory system. In employing this method it is absolutely essential that the animals should not have been bled before.